ATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
BO 43425		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IL99/00396	19/07/1999	20/07/1998
International Patent Classification (IPC) or national classification and IPC C12N15/82		
Applicant STATE OF ISRAEL-MINISTRY OF AGRICULTURE et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
16/02/2000	15.11.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Paresce, D Telephone No. +49 89 2399 8995

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IL99/00396

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-15 as originally filed

Claims, No.:

1-30 as originally filed

Drawings, sheets:

1/1 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 1-10, 17-19.

because:

☒ the said international application, or the said claims Nos. 1-10, 17-19 relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N) Yes: Claims 11-16, 20-22, 24, 26, 28-30

	No:	Claims	23, 25, 27
Inventive step (IS)	Yes:	Claims	11-16, 20-22, 24, 26, 28
	No:	Claims	29-30
Industrial applicability (IA)	Yes:	Claims	11-16, 20-30
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item I

Basis of the report

This written opinion will be based on claims 11-16, 20-30 which are directed to a method of producing genetically transformed plants which have elevated starch content comprising the use of the *Lycopersicon hirsutum*-derived large subunit (LS1) of ADPGPPase.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The subject-matter of present claims 1-10, 17-19 reads on to essentially biological processes for the production of plants (i.e. crossing). Thus these claims relate to methods and plant varieties considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the novelty, inventive step and industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1) The documents mentioned in this communication are numbered as in the search report, i.e. D1 corresponds to the first document of the search report.

2) **Novelty: Article 33(2) PCT**

The subject-matter of claims 23, 25, 27 is not considered new in the sense of Article 33(2) PCT for the following reasons:

D1 describes a chromosomal segment from the wild tomato, *Lycopersicon chmielewskii*, introgressed into the *Lycopersicon esculentum genome*, which was mapped to chromosome 7 and which was found to be associated with higher mature green fruit starch concentration and red ripe fruit weight. D1 describes the

physiological mechanisms controlled by said segment, called 7T, on tomato soluble solids and pH and its genetic regulation during fruit development. The effects of 7T were studied in backcross inbred lines developed from a cross between a *Lycopersicon esculentum* line and a processing cultivar (D1, see abstract and materials and methods).

D7 discloses a method for breeding tomato plants that produce tomatoes having superior taste characteristics including the steps of, crossing at least one *Lycopersicon esculentum* plant with a *Lycopersicon spp.* to produce hybrid seeds, collecting the hybrid F_1 seeds, growing plants from the F_1 seeds, pollinating the F_1 plants, collecting the hybrid seeds produced by the F_1 plants, growing plants from the seeds produced by the F_1 plants, measuring sucrose, glucose and fructose content of ripe fruit produced from the plants grown from the seeds of the F_1 plants, and selecting plants with tomato fruits having desired characteristics (see abstract).

Therefore, the subject-matter of claims 23, 25, 27 is not considered new in the sense of Article 33(2) PCT. The subject-matter of claims 11-16, 20-22, 24, 26, 28-30 has not been made available to the public by any of the available prior art documents and can therefore be regarded as novel.

2) **Inventive Step: Article 33(3) PCT**

The subject-matter of claims 11-16, 20-22, 24, 26, 28 cannot be derived from the available prior art in an obvious manner and therefore complies with the requirements of Article 33(3) PCT. The subject-matter of claims 29-30, however, is not considered to involve an inventive step in the sense of Article 33(3) PCT for the following reasons:

The closest prior art to evaluate the inventiveness of these claims is D9.

Claim 29 is directed to a gene that controls sucrose-starch metabolism comprising a specific sequence. Claim 30 is directed to a protein that controls sucrose-starch metabolism comprising a specific amino acid sequence. The IPEA considers that because of the term "comprising", the scope of these claims includes not only the

large subunit of ADPGPPase but longer sequences as well. In particular, the subject-matter of these claims includes the full-length sequence of the ADPGPPase gene or protein.

The enzyme ADPGPPase (EC 2.7.7.27) is known in the prior art and is known to be involved in starch synthesis in higher plants (see D9). Furthermore, D9 describes the molecular cloning and organ-specific expression of three isoforms of tomato ADPGPPase large subunits. Three cDNAs encoding different isoforms of ADPGPPase large subunits from tomato plants were isolated and the deduced amino acid sequences are shown in figure 2. The amino acid sequence of AGPL1 shown in D9 is 97.88% identical to the sequence of claim 30.

Therefore, the subject-matter of 29-30 consists in the provision of amino acid and DNA sequences that are only slightly different from those of D9. These variations are considered to come within the scope of the customary practice followed by persons skilled in the art. These amino acid and DNA sequences claimed in the present application can only be regarded as inventive, if the protein encoded by said sequences presented unexpected effects or properties in relation to the other proteins disclosed in D9. However, no such effects or properties are indicated in the application. Therefore, an inventive step for the claimed protein cannot at present be recognized, unless said protein will show some kind of unexpected advantages over those described in prior art, which should be demonstrated.

VIII. Certain observations on the international application

1) Clarity: Article 6 PCT

Article 6 PCT requires amongst other things that the claims, which define the matter for which protection is sought (i.e. the object of invention) be clear. This has to be interpreted as meaning not only that a claim from a technical point of view must be comprehensible, but also that it must define clearly the object of the invention, that is to say, it must indicate all the essential features thereof. The essential features are regarded as all features which are necessary to obtain the desired effect, or differently expressed, those features which are necessary to solve the technical problem with which the application is concerned. In other

words, all technical features which enable the skilled person to put the claimed matter into practice without undue burden i.e. without experimentation or without application of inventive skill.

Claim 11 is not clear because the expression, "the above described ADPGPPase LS1 protein" is not clear. Given the high homology between the ADPGPPase large subunits disclosed in D9 and the ADPGPPase LS1 protein of the present application, the IPEA feels it is necessary to more precisely define the "ADPGPPase LS1 protein". At present, the technical features given in claim 11 do not allow one to distinguish the protein of the present application with the protein described in D9. The feature, "*Lycopersicon hirsutum*-derived" is not a limiting feature which would render the enzyme novel over prior art. The fact that a product is produced by means of a new process does not render this product novel. The protein subunit should be clearly and unambiguously characterized e.g. by reference to technical features, (i.e. function and sequence information).

Furthermore, the plants, fruits or seeds referred to in claims 24, 26, and 28 are not clearly defined. The fact that a product is produced by means of a new process does not render this product novel. The plants, fruits or seeds should be clearly and unambiguously characterized e.g. by reference to technical features, in order to satisfy the requirements of Article 6 PCT.

ATENT COOPERATION TREAT

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
34857		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/IL 99/ 00396	19/07/1999	20/07/1998

Applicant

STATE OF ISRAEL-MINISTRY OF AGRICULTURE et al.

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [X] furnished subsequently to this Authority in written form.

   [X] furnished subsequently to this Authority in computer readble form.

   [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of Invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. –

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL 99/00396

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

[redacted]national Application No
PCT/IL 99/00396

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/82 C12N15/54 C12N9/12 C12N5/10 C12Q1/68
A01H1/04 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AZANZA F ET AL: "Genes from Lycopersicon chmielewskii affecting tomato quality during fruit ripening." THEORETICAL AND APPLIED GENETICS 1995, vol. 91, no. 3, August 1995 (1995-08), pages 495-504, XP000910662 ISSN: 0040-5752 the whole document --- -/--	1,5, 17-19, 23,27

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 May 2000	14/06/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Holtorf, S

2

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	DINAR M ET AL: "THE RELATIONSHIP BETWEEN STARCH ACCUMULATION AND SOLUBLE SOLIDS CONTENT OF TOMATO LYCOPERSICON-ESCULENTUM FRUITS" JOURNAL OF THE AMERICAN SOCIETY FOR HORTICULTURAL SCIENCE 1981, vol. 106, no. 4, 1981, pages 415-418, XP000911193 ISSN: 0003-1062 the whole document ---	1-30
A	STARK DAVID M ET AL: "Improvement of food quality traits through enhancement of starch biosynthesis." CONFERENCE;LEXINGTON, KENTUCKY, USA; OCTOBER 1-4, 1995, vol. 792, 1996, pages 26-36, XP000911159 Annals of the New York Academy of Sciences Annals of the New York Academy of Sciences;Engineering plants for commercial products and applications. 1996 New York Academy of Sciences 2 East 63rd Street, New York, New York 10021, USA ISBN: 1-57331-047-6 ---	1-30
A	SCHAFFER ARTHUR A ET AL: "Sucrose-to-starch metabolism in tomato fruit undergoing transient starch accumulation." PLANT PHYSIOLOGY (ROCKVILLE) 1997, vol. 113, no. 3, 1997, pages 739-746, XP002137704 ISSN: 0032-0889 cited in the application page 745 ---	1-30
A	SCHAFFER ARTHUR A ET AL: "Modification of carbohydrate content in developing tomato fruit." 94TH ANNUAL INTERNATIONAL CONFERENCE OF THE AMERICAN SOCIETY FOR HORTICULTURAL SCIENCE;SALT LAKE CITY, UTAH, USA; JULY 23-26, 1997, vol. 32, no. 3, 1997, page 551 XP000910547 Hortscience 1997 ISSN: 0018-5345 the whole document --- -/--	1-30

2

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	MIRON D ET AL: "SUCROSE PHOSPHATE SYNTHASE SUCROSE SYNTHASE AND INVERTASE ACTIVITIES IN DEVELOPING FRUIT OF LYCOPERSICON-ESCULENTUM MILL. AND THE SUCROSE ACCUMULATING LYCOPERSICON-HIRSUTUM HUMB. AND BONPL" PLANT PHYSIOLOGY (BETHESDA) 1991, vol. 95, no. 2, 1991, pages 623-627, XP002137705 ISSN: 0032-0889 cited in the application the whole document	1-30
A	WO 94 22289 A (SCHAFFER ARTHUR ;PERI DEV APPLIC 1985 LTD (IL)) 13 October 1994 (1994-10-13) the whole document	1-30
A	WO 92 14831 A (SALK INST BIOTECH IND) 3 September 1992 (1992-09-03) the whole document	1-30
A	PARK S -W ET AL: "Molecular cloning and organ-specific expression of three isoforms of tomato ADP-glucose pyrophosphorylase gene" GENE: AN INTERNATIONAL JOURNAL ON GENES AND GENOMES,GB,ELSEVIER SCIENCE PUBLISHERS, BARKING, vol. 206, no. 2, 12 January 1998 (1998-01-12), pages 215-221, XP004108898 ISSN: 0378-1119 the whole document	30
A	HADAS R ET AL: "PCR-generated molecular markers for the invertase gene and sucrose accumulation in tomato." THEORETICAL AND APPLIED GENETICS 1995, vol. 90, no. 7-8, 1995, pages 1142-1148, XP000910663 ISSN: 0040-5752 the whole document	1-30
A	WO 91 19806 A (MONSANTO CO) 26 December 1991 (1991-12-26) page 9,59	1-30

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
T	SCHAFFER ARTHUR A ET AL: "ADPglucose pyrophosphorylase activity and starch accumulation in immature tomato fruit: The effect of a Lycopersicon hirsutum-derived introgression encoding for the large subunit." PLANT SCIENCE (SHANNON). MARCH 21, 2000, vol. 152, no. 2, 21 March 2000 (2000-03-21), pages 135-144, XP000910545 ISSN: 0168-9452 the whole document ---	
T	SCHAFFER ARTHUR A ET AL: "Modification of carbohydrate content in developing tomato fruit." HORTSCIENCE OCT., 1999, vol. 34, no. 6, October 1999 (1999-10), pages 1024-1027, XP000910546 ISSN: 0018-5345 page 1024, right column; page 1025, page 1026; right column -----	

INTERNATIONAL SEARCH REPORT

[redacted]ation on patent family members

[redacted]national Application No
PCT/IL 99/00396

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9422289 A	13-10-1994	AU 6496294 A	24-10-1994
		US 5817913 A	06-10-1998
WO 9214831 A	03-09-1992	AU 1456292 A	15-09-1992
		BR 9205480 A	01-03-1994
		EP 0573566 A	15-12-1993
		HU 66831 A	30-01-1995
		MX 9200751 A	01-09-1992
		US 5576428 A	19-11-1996
		US 5665579 A	09-09-1997
WO 9119806 A	26-12-1991	AU 644203 B	02-12-1993
		AU 8220291 A	07-01-1992
		CA 2081885 A	19-12-1991
		EP 0536293 A	14-04-1993
		FI 925228 A	18-11-1992
		NO 924893 A	11-02-1993
		US 5498830 A	12-03-1996
		US 5608149 A	04-03-1997

TENT COOPERATION TREA Y

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 01 May 2000 (01.05.00)	
International application No. PCT/IL99/00396	***Applicant's or agent's file reference*** 34857
International filing date *(day/month/year)* 19 July 1999 (19.07.99)	***Priority date*** *(day/month/year)* 20 July 1998 (20.07.98)
Applicant SCHAFFER, Arthur, A. *et al*	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

16 February 2000 (16.02.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Pascal Piriou

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

U 013220-5

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

29 NOV 2000

PCT

To:

DE BRUIJN, Leendert C.
Nederlandsch Octrooibureau
Scheveningseweg 82
P.O. Box 29720
NL-2502 LS The Hague
PAYS-BAS

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	15.11.2000
Applicant's or agent's file reference	**IMPORTANT NOTIFICATION**
BO 43425	

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IL99/00396	19/07/1999	20/07/1998

Applicant
STATE OF ISRAEL-MINISTRY OF AGRICULTURE et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Vullo, C Tel. +49 89 2399-8061

Form PCT/IPEA/416 (July 1992)

ATENT COOPERATION TREATY

U 013220-5

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
BO 43425		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IL99/00396	19/07/1999	20/07/1998
International Patent Classification (IPC) or national classification and IPC C12N15/82		
Applicant STATE OF ISRAEL-MINISTRY OF AGRICULTURE et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
16/02/2000	15.11.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Paresce, D Telephone No. +49 89 2399 8995

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IL99/00396

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-15 as originally filed

Claims, No.:

1-30 as originally filed

Drawings, sheets:

1/1 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 1-10, 17-19.

because:

☒ the said international application, or the said claims Nos. 1-10, 17-19 relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N) Yes: Claims 11-16, 20-22, 24, 26, 28-30

	No:	Claims	23, 25, 27
Inventive step (IS)	Yes:	Claims	11-16, 20-22, 24, 26, 28
	No:	Claims	29-30
Industrial applicability (IA)	Yes:	Claims	11-16, 20-30
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item I

Basis of the report

This written opinion will be based on claims 11-16, 20-30 which are directed to a method of producing genetically transformed plants which have elevated starch content comprising the use of the *Lycopersicon hirsutum*-derived large subunit (LS1) of ADPGPPase.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The subject-matter of present claims 1-10, 17-19 reads on to essentially biological processes for the production of plants (i.e. crossing). Thus these claims relate to methods and plant varieties considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the novelty, inventive step and industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1) The documents mentioned in this communication are numbered as in the search report, i.e. D1 corresponds to the first document of the search report.

2) **Novelty: Article 33(2) PCT**

The subject-matter of claims 23, 25, 27 is not considered new in the sense of Article 33(2) PCT for the following reasons:

D1 describes a chromosomal segment from the wild tomato, *Lycopersicon chmielewskii*, introgressed into the *Lycopersicon esculentum genome*, which was mapped to chromosome 7 and which was found to be associated with higher mature green fruit starch concentration and red ripe fruit weight. D1 describes the

physiological mechanisms controlled by said segment, called 7T, on tomato soluble solids and pH and its genetic regulation during fruit development. The effects of 7T were studied in backcross inbred lines developed from a cross between a *Lycopersicon esculentum* line and a processing cultivar (D1, see abstract and materials and methods).

D7 discloses a method for breeding tomato plants that produce tomatoes having superior taste characteristics including the steps of, crossing at least one *Lycopersicon esculentum* plant with a *Lycopersicon spp.* to produce hybrid seeds, collecting the hybrid F_1 seeds, growing plants from the F_1 seeds, pollinating the F_1 plants, collecting the hybrid seeds produced by the F_1 plants, growing plants from the seeds produced by the F_1 plants, measuring sucrose, glucose and fructose content of ripe fruit produced from the plants grown from the seeds of the F_1 plants, and selecting plants with tomato fruits having desired characteristics (see abstract).

Therefore, the subject-matter of claims 23, 25, 27 is not considered new in the sense of Article 33(2) PCT. The subject-matter of claims 11-16, 20-22, 24, 26, 28-30 has not been made available to the public by any of the available prior art documents and can therefore be regarded as novel.

2) **Inventive Step: Article 33(3) PCT**

The subject-matter of claims 11-16, 20-22, 24, 26, 28 cannot be derived from the available prior art in an obvious manner and therefore complies with the requirements of Article 33(3) PCT. The subject-matter of claims 29-30, however, is not considered to involve an inventive step in the sense of Article 33(3) PCT for the following reasons:

The closest prior art to evaluate the inventiveness of these claims is D9.

Claim 29 is directed to a gene that controls sucrose-starch metabolism comprising a specific sequence. Claim 30 is directed to a protein that controls sucrose-starch metabolism comprising a specific amino acid sequence. The IPEA considers that because of the term "comprising", the scope of these claims includes not only the

large subunit of ADPGPPase but longer sequences as well. In particular, the subject-matter of these claims includes the full-length sequence of the ADPGPPase gene or protein.

The enzyme ADPGPPase (EC 2.7.7.27) is known in the prior art and is known to be involved in starch synthesis in higher plants (see D9). Furthermore, D9 describes the molecular cloning and organ-specific expression of three isoforms of tomato ADPGPPase large subunits. Three cDNAs encoding different isoforms of ADPGPPase large subunits from tomato plants were isolated and the deduced amino acid sequences are shown in figure 2. The amino acid sequence of AGPL1 shown in D9 is 97.88% identical to the sequence of claim 30.

Therefore, the subject-matter of 29-30 consists in the provision of amino acid and DNA sequences that are only slightly different from those of D9. These variations are considered to come within the scope of the customary practice followed by persons skilled in the art. These amino acid and DNA sequences claimed in the present application can only be regarded as inventive, if the protein encoded by said sequences presented unexpected effects or properties in relation to the other proteins disclosed in D9. However, no such effects or properties are indicated in the application. Therefore, an inventive step for the claimed protein cannot at present be recognized, unless said protein will show some kind of unexpected advantages over those described in prior art, which should be demonstrated.

VIII. Certain observations on the international application

1) Clarity: Article 6 PCT

Article 6 PCT requires amongst other things that the claims, which define the matter for which protection is sought (i.e. the object of invention) be clear. This has to be interpreted as meaning not only that a claim from a technical point of view must be comprehensible, but also that it must define clearly the object of the invention, that is to say, it must indicate all the essential features thereof. The essential features are regarded as all features which are necessary to obtain the desired effect, or differently expressed, those features which are necessary to solve the technical problem with which the application is concerned. In other

words, all technical features which enable the skilled person to put the claimed matter into practice without undue burden i.e. without experimentation or without application of inventive skill.

Claim 11 is not clear because the expression, "the above described ADPGPPase LS1 protein" is not clear. Given the high homology between the ADPGPPase large subunits disclosed in D9 and the ADPGPPase LS1 protein of the present application, the IPEA feels it is necessary to more precisely define the "ADPGPPase LS1 protein". At present, the technical features given in claim 11 do not allow one to distinguish the protein of the present application with the protein described in D9. The feature, "*Lycopersicon hirsutum*-derived" is not a limiting feature which would render the enzyme novel over prior art. The fact that a product is produced by means of a new process does not render this product novel. The protein subunit should be clearly and unambiguously characterized e.g. by reference to technical features, (i.e. function and sequence information).

Furthermore, the plants, fruits or seeds referred to in claims 24, 26, and 28 are not clearly defined. The fact that a product is produced by means of a new process does not render this product novel. The plants, fruits or seeds should be clearly and unambiguously characterized e.g. by reference to technical features, in order to satisfy the requirements of Article 6 PCT.

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(71) Applicant *(for all designated States except US)*: STATE OF ISRAEL–MINISTRY OF AGRICULTURE [IL/IL]; Volcani Research Center, P.O. Box 6, 50250 Beit Dagan (IL).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: SCHAFFER, Arthur, A. [IL/IL]; Hazayit Street 16, 73127 Hashmonaim (IL). LEVIN, Ilan [IL/IL]; Eshel Street 8, 76804 Mazkeret Batya (IL). PETREIKOV, Marina [IL/IL]; Bernstein Street 55/22, 75000 Rishon le Zion (IL). BAR, Moshe [IL/IL]; Nahal Soreq Street 10, 75246 Rishon le Zion (IL).

(74) Agents: COLB, Sanford, T. et al.; Sanford T. Colb & Co., P.O. Box 2273, 76122 Rehovot (IL).

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(54) Title: CONTROLLING STARCH SYNTHESIS

(57) Abstract

A method for controlling starch synthesis in tomatoes including providing a population of plants derived from interspecific crosses of *Lycopersicon* spp. with *Lycopersicon esculentum* genotypes, and selecting individuals of the population that each contain an allele of a gene that increases starch synthesis, the gene originating from the *Lycopersicon* spp.

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CONTROLLING STARCH SYNTHESIS

FIELD OF THE INVENTION

The present invention relates to a method of breeding tomatoes with increased starch content in the young fruit and subsequently increased soluble solids content in the mature fruit. In addition, it relates to the use of genes that increase starch in the tomato.

BACKGROUND OF THE INVENTION

The solids content of ripe tomato fruit is a major determinant of its quality. Increasing the soluble solids (largely sugars and organic acids) content and thereby improving the value of industry tomatoes and the taste of fresh market tomatoes have been the goal of research projects for many years. Several approaches to improving solids levels have been taken, encompassing both agrotechnical and genetic manipulations.

Soluble solids content of tomato fruit are primarily comprised of sugars, organic acids and salts. Collectively the soluble solids content is a major determinant of fruit quality, both for industry use and for fresh market consumption. Approximately half of the soluble solids content is contributed by the sugar fraction which, in all standard cultivars of *Lycopersicon esculentum*, consists of the monosaccharide reducing sugars glucose and fructose in approximately equimolar concentrations.

Several strategies to increase sugar concentration in ripe tomato fruit have been explored. Genetic manipulations include the transfer of undefined traits of high soluble solids from wild species of Lycopersicon (Rick C.M. 1974. Hilgardia 42:493-510; and Hewitt J.D., Dinar M. and Stevens M.A. 1982. J. Am. Soc. Hort. Sci. 107:896-900) and more recently the transfer of the genetic trait of sucrose accumulation from the wild *Lycopersicon chmielewskii* (Yelle S., Hewitt J.D., Robinson N.L., Damon N.S. and Bennett A.B. 1988. Pl. Physiol. 87:737-740; and Yelle S., Chetelat R.T., Dorais M., Deverna J.W. and Bennett A.B. 1991. Pl. Physiol. 95:1026-1035.) and *L. hirsuum* (Miron D. and Schaffer A.A. 1991. Pl. Physiol. 95:623-627), as well as the transfer of the genetic trait of high fructose to glucose ratio in the mature fruit, from *L. hirsutum* (US Patent Application 08/530,216, the disclosure of which is incorporated herein by reference). The latter approach was made possible by the study of the components of carbohydrate metabolism in developing tomato fruit tissue with the purpose of identifying biochemical steps whose modification may lead to increased soluble carbohydrate content in the fruit (Yelle et al., 1988, 1991; Miron and Schaffer, 1991). Once identified, these biochemical processes could then be targeted for modification by classical genetic means, assisted by selection for the genotypic biochemical trait, or by molecular genetic strategies.

The young, developing tomato fruit is characterized by a transient starch accumulation which can contribute over 25% of the dry weight of the fruit tissue. Starch concentration begins to increase within days after anthesis and reaches peak concentrations before the mature green stage (Schaffer, A.A. and Petreikov, M. 1997a. Plant Physiology 113:739-746). At the mature stage this starch is practically absent in the tomato fruit tissue. It has been hypothesized that the transiently accumulated starch serves as a reservoir of carbohydrate for the later accumulation of soluble sugars in the mature fruit (Dinar M. and Stevens M.A. 1981. J. Am. Soc. Hort. Sci. 106:415-418). Dinar and Stevens laid the groundwork for this hypothesis in their study comparing seven genotypes of tomato whose total soluble solids (TSS) values in the ripe fruit spanned the spectrum from 4.6 to 6.3 °Brix. They found that TSS values in ripe fruit were positively correlated with starch content in young, immature fruit and proposed that the products of starch hydrolysis contribute to the accumulation of soluble sugars.

The tomato plant translocates photosynthate to the fruit in the form of sucrose (Walker L.J. and Ho L.C. 1977. Ann. Bot. 41:813-823) and therefore, the temporal accumulation of starch will presumably be determined by temporal changes in the activities of key enzymes involved in sucrose to starch metabolism. The enzymatic pathway of starch synthesis in young tomato fruit has been studied and described (Schaffer, A.A. and Petreikov, M. 1997a. Plant Physiology 113:739-746; Schaffer, A.A. and Petreikov, M. 1997b. Physiologia Plantarum 101:800-806). Four enzymes were identified that potentially limit starch accumulation in these fruit, based on their absolute activities, as well as on the developmental changes in their activities which correlate temporally with the developmental changes in starch levels. These enzymes include those that catalyze the initial steps of sucrose metabolism in the young fruit (sucrose synthase, E.C. 2.4.1.13, and fructokinase, E.C. 2.7.1.4) as well as the latter steps of starch synthesis (ADP-glucose pyrophosphorylase, E.C. 2.7.7.27, and starch synthase, E.C., 2.4.1.21). In addition, Schaffer and Petreikov have shown that starch accumulation is tissue specific, localized primarily in the columella and inner pericarp tissues, and suggested that relative contributions of these tissues to fruit bulk could impact on fruit starch content.

Research has clearly shown that one of the above mentioned enzymes, ADPGPPase (ADP-glucose pyrophosphorylase), may be limiting to starch synthesis in tomato fruit, as well as in other starch accumulating tissues, such as potato tubers. In Stark D.M., Barry G.F., and Kishore G.M. 1996. Ann. NY Cad Sci 792:26-36, transgenic tomato plants and potato plants were developed with a bacterial mutant form of ADPGPPase (*E. coli*, GlgC16, a glycogen overproducer). Transgenic tomatoes showed a higher starch content in the immature fruit and

an increased sugar content in the mature fruit. Transgenic potato tubers with the same bacterial gene construct also showed an increase in starch content. Reciprocally, inhibition of ADPGPPase activity decreased the starch content of transgenic potato tubers, further indicating the importance of ADPGPPase in controlling starch accumulation.

The use of a gene for ADPGPPase of bacterial origin requires molecular genetic manipulations in order for the gene to function in eucaryotic plant tissue. For example, it requires that an artificial gene construct be developed that will encode a fusion polypeptide containing a specific amino terminal transit peptide, not present in the procaryotic gene, as well as other DNA sequence additions that will cause in plant cells transcriptional termination, and the addition of polyadenylated nucleotides to the 3' end of the RNA sequence. In comparison, the use of a plant gene for similar transformations does not require these manipulations.

In addition, the development of plants with increased or modified activity of these enzymes, based on the natural transfer through classical breeding techniques of naturally occurring alleles of these genes, can benefit from a number of advantages. For example, classical breeding techniques lead to the positioning of the desired allele in the natural position of the gene of interest, leading to genetic stability and obviating the unpredictable "position" effects characteristic of the development of transgenic organisms. In addition, with respect to consumer preferences, there are obvious advantages of a naturally derived commercial product such as a tomato fruit, compared to a transgenically derived tomato fruit.

With respect to fructokinase, two genes from tomato fruit have been identified, cloned and sequenced (Kanayama, Y. et al. 1997. Plant Physiology 113:1379-1384). One of these genes, FK2, is particularly involved in the metabolic pathway associated with starch synthesis (Kanayama et al. 1998. Plant Physiology 117:85-90). Similarly, the gene for sucrose synthase from tomato fruit has been cloned and sequenced (Wang, F., et al. Plant Physiology 103:1463-1464;) and has been shown to be the gene for sucrose synthase of sink tissue (Fu, H. and Park, W.D. Plant Cell 7:1369-1385).

With respect to ADPGPPase, the enzyme functions in higher plants as a heterotetramer, comprised of two large and two small subunits (Preiss, J. and Sivak, M. In: Photoassimilate Distribution in Plants and Crops, Zamski, E. and Schaffer, A.A., eds., Marcel Dekker Publ, NYC, pp.63-96, 1996) which are under independent genetic control. Three separate *L. esculentum* genes coding for the large subunits and one gene for the small subunit have recently been cloned and sequenced (Chen, B.Y. and Janes, H.,1995, Plant Physiology 109:1498; Park, S.W. and Chung, W.I. 1998. Gene 206:215-221). Much effort has been made

in order to identify sources of ADPGPPase genes in plants that may contribute to improving starch content, as for example in corn (Giroux, M.J. et al., Proc. Natl. Acad. Sci. USA 93:5824-5829), where site-specific mutation of the gene for the large subunit of ADPGPPase, using a transposable element *Ds* system, led to an insertion mutation of ADPGPPase which had decreased sensitivity to the ADPGPPase inhibitor, phosphate, as well as increased seed weight.

As regards to the use of wild species of *Lycopersicon* for the modification of carbohydrate metabolism in tomatoes, as described in US Patent *Application* 08/530,216, although the fructose to glucose ratio in *L. hirsutum* is high, the actual amount of fructose and glucose is very low. Recombination of the genetic trait of fructose to glucose ratio, together with the trait of high glucose and fructose levels from *L. esculentum* yielded the unobvious and desirable trait of high levels of hexose, together with the high ratio of fructose to glucose. However, *L. hirsutum* fruit accumulate only low amounts of starch, as compared to the cultivated *L. esculentum* (Miron and Schaffer, 1991, Plant Physiology 95:623-627). Similarly, other wild species of *Lycopersicon* also accumulate little starch (i.e., *L. chmieliewskii*, Yelle et al. 1988. Plant Physiology 87:737-740). Thus, the prior art has never expected or considered the use of wild tomatoes as a possible source of genetic variability for the increase in starch accumulation.

SUMMARY OF THE INVENTION

The present invention seeks to provide selection strategies for tomatoes with high starch content in the young fruit and subsequent high soluble solids in the mature fruit.

There is thus provided in accordance with a preferred embodiment of the present invention a method for controlling starch synthesis in tomatoes including providing a population of plants derived from interspecific crosses of *Lycopersicon* spp. with *Lycopersicon esculentum* genotypes, and selecting individuals of the population that each contain an allele of a gene that increases starch synthesis, the gene originating from the *Lycopersicon* spp.

In accordance with a preferred embodiment of the present invention the step of selecting includes selecting individuals that each contain the allele of the gene that encodes for an enzyme that catalyzes a metabolic step in starch synthesis.

Further in accordance with a preferred embodiment of the present invention the step of selecting includes selecting individuals that each contain the allele of the gene that encodes for a subunit of ADPGPPase.

Still further in accordance with a preferred embodiment of the present invention the step of selecting includes selecting individuals that each contain the allele of the gene that

encodes for a *Lycopersicon hirsutum*-derived subunit of ADPGPPase.

Additionally in accordance with a preferred embodiment of the present invention the step of selecting includes selecting by using a molecular marker for the gene.

In accordance with a preferred embodiment of the present invention the molecular marker includes step of selecting includes a *Lycopersicon hirsutum*-derived large subunit (LS1) of ADPGPPase.

Further in accordance with a preferred embodiment of the present invention the step of selecting includes selecting by measuring activity of the enzyme in young fruit and selecting those young fruit with high activity of the enzyme.

Still further in accordance with a preferred embodiment of the present invention the step of selecting includes selecting by measuring ADPGPPase activity of the young fruit, and selecting those young fruit with high ADPGPPase activity.

In accordance with a preferred embodiment of the present invention the *Lycopersicon* spp. includes a *Lycopersicon* spp. of green-fruited *Eriopersicon* subgenus. Preferably the *Lycopersicon* spp. includes *Lycopersicon hirsutum*.

There is also provided in accordance with a preferred embodiment of the present invention a method of producing genetically transformed plants which have elevated starch content, including the steps of inserting into the genome of a plant cell a recombinant double stranded DNA molecule including a selected promoter, a structural DNA sequence that causes the production of an RNA sequence which encodes the above described ADPGPPase LS1 protein, obtaining transformed plant cells, and regenerating from the transformed plant cells genetically transformed plants with elevated starch content.

In accordance with a preferred embodiment of the present invention the plant cell is selected from the group consisting of a tomato cell, a potato cell, a cell from a solanaceous plant, a legume cell, and a grain crop cell.

Further in accordance with a preferred embodiment of the present invention the promoter is selected from the group consisting of an immature fruit promoter, a tuber promoter, and a seed promoter.

Still further in accordance with a preferred embodiment of the present invention the step of regenerating includes regenerating genetically transformed plants with elevated starch content in an immature fruit.

In accordance with a preferred embodiment of the present invention the step of regenerating includes regenerating genetically transformed plants with elevated starch content

in a tuber.

Further in accordance with a preferred embodiment of the present invention the step of regenerating includes regenerating genetically transformed plants with elevated starch content in a seed.

Still further in accordance with a preferred embodiment of the present invention the methods of the present invention also include the step of propagating the individuals of the population or the genetically transformed plants. The propagating may be by vegetative propagation or by seed, for example.

There are also provided in accordance with a preferred embodiment of the present invention a plant produced according to any of the methods of the present invention, a fruit produced by such a plant, and a seed which when grown yields such a plant.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention will be understood and appreciated more fully from the following detailed description, taken in conjunction with the drawing in which:

Figure 1 is a histogram of TSS (total soluble solids) values from individual plants of three BCF6 lines (95-929, 95-931 and 95-935), compared to a standard cultivar, M-82. Data from each plant is an average of TSS values from 5 individual fruit. Single plant selections from 95-929, 95-931 and 95-935 led to the BCF7 high starch breeding lines 900, 901 and 904, respectively.

In addition, the following tables are presented:

Table 1 shows the starch levels and activity of enzymes involved in the metabolism of sucrose to starch in young tomato fruit of the breeding lines 900, 901 and 904, compared to the standard cultivar, M-82. The * signifies statistical difference between each individual high starch line when compared to M-82 and does not indicate differences between the high starch lines. For the enzymes PGI (phosphoglucosisomerase), PGM (phosphoglucomutase) and UDPGPPase only one fruit was analyzed per line and since enzyme activity in all lines was relatively high and apparently in excess (as in Schaffer and Petreikov, 1997a) no significant differences were assumed. For the other assays, a minimum of 4 fruit from individual plants were assayed.

Table 2 shows the TSS values of mature fruit, and the starch levels of immature fruit of M-82, 904, the hybrid between them, a mix of 11 hybrids between 904 and 11 introgression lines (described in text), and a mix of the 11 parallel hybrids between M-82 and the same 11 introgression lines. At least two fruit from each of the individual hybrids were measured and

the average represents accordingly a minimum of 22 individual analyses. At least three fruit from each of M-82, 904 and the hybrid between them were assayed.

Table 3 shows the enzyme activities of immature fruit pericarp of M-82, 904, the hybrid between them, a mix of 6 of the 11 hybrids between 904 and 11 introgression lines (described in text), and the parallel mix of 6 of the 11 hybrids between M-82 and the same introgression lines. For M-82, 904 and the hybrid between them, two fruit from individual plants were assayed.

Table 4 shows the nucleotide sequences of the forward and reverse primers used in the PCR analysis of the 3 large and 1 small subunits of ADPGPPase and the restriction endonucleases used to digest the PCR product in order to obtain the *L. hirsutum* specific allele.

Table 5 shows the activity levels of ADPGPPase of F2 plants from the cross of line 904 and M-82. The LS1 genotype of the plants was characterized at the seedling stage, as described further herein. ADPGPPase activity and starch levels are the averages from 4 fruit (8-13 gr.) from individual F2 plants. TSS values are the average of a minimum of 5 fruit of each genotype.

Table 6 is the nucleotide sequence of ADPGPPase LS1 (ADPGlucose pyrophosphorylase, large subunit 1) from *L. hirsutum*.

Table 7 is the derived amino acid sequence for ADPGPPase LS1 (ADPGlucose pyrophosphorylase, large subunit 1) from *L. hirsutum*.

DETAILED DESCRIPTION OF A PREFERRED EMBODIMENT

The following is one example of carrying out the present invention. Plants of the *L. esculentum* breeding line 1630 (a Volcani Institute male sterile breeding line, used to simplify the production of the interspecific hybrid) were pollinated with pollen of the wild species *L. hirsutum* (LA1777). Hybrid F1 plants were grown and allowed to self pollinate, generating F2 seed. F2 seed were sown and about 350 plants were grown in a screenhouse and allowed to self pollinate.

Ripe fruit from each individual plant which produced fruit were individually analyzed for soluble solids (refractometrically). Only 25 of the interspecific F2 plants freely produced fruit. Pollen from one-plant (F2-82) which was characterized by high soluble sugar level in the mature fruit (71 mg soluble sugar, composed of sucrose, glucose and fructose, per gram fresh weight of fruit) was used to pollinate a standard, industry type tomato (breeding line A701) for the production of the backcross-F1 (BC-F1) population. 100 BC-F1 plants were grown in the field and mature fruit of individual plants were analyzed for soluble solids, refractometrically,

as well as soluble sugars, as above. A pedigree, single seed descent selection program was carried out, selecting the plants with highest total soluble solids and soluble sugar levels. Each generation consisted of at least 100 plants. This selection technique was carried out for six generations, until the BC-F7 generation, leading to breeding lines with higher solids levels than the standard industry type cultivars.

Fig. 1 shows a series of histograms representing the BCF6 lines from which three BCF7 breeding lines were selected. The BCF6 95-929 had an average TSS value of 4.8 (11 plants, 5 fruits per plant), the BCF6 95-931 had an average TSS value of 5.7 (8 plants, 5 fruits per plant) and the BCF6 95-935 had an average TSS value of 6.1 (15 plants, 5 fruits per plant), as compared to the standard cultivar, M-82 which had an average TSS value of 3.5 (10 plants, 5 fruits per plant). The individual plant selection 95-929-6, which led to the BCF7 line 900, had a TSS of 5.5 with a plant yield of 9.1 kg fruit. The individual plant selection 95-931-2, which led to the BCF7 line 901, had a TSS of 6.5 with a plant yield of 7.2 kg fruit. The individual plant selection 95-935-5, which led to the BCF7 line 904, had a TSS of 6.6 with a plant yield of 4.7 kg fruit. The average plant yield of M-82 was 6.1 kg, based on an average of 6 plants.

In the BC-F7 generation immature fruit (approx. 15 days after anthesis) were measured for starch levels, as described in Schaffer and Petreikov (1997a). Lines 900, 901 and 904 were characterized by immature starch levels significantly higher than that of a standard industry type tomato cultivar, M-82 (Table 1). A comparative survey of enzymatic activities involved in sucrose to starch metabolism, as described in Schaffer and Petreikov (1997a), was performed on immature fruit of the two breeding lines and the standard, M-82. Typical results are presented in Table 1 and show that breeding line 900 is characterized by significantly higher levels of activity of the enzymes ADPGPPase and fructokinase while lines 901 and 904 are characterized by significantly higher activities of the enzyme ADPGPPase alone. Line 904 is characterized by the highest levels of the enzyme ADPGPPase among the lines we studied and was used for further study of the role of ADPGPPase in starch accumulation and TSS levels of tomato fruit.

The high starch line 904 was further hybridized with eleven independent tomato breeding lines. In parallel, the standard industry type tomato cultivar, M-82, was similarly hybridized with each of these eleven lines. The eleven lines used were from the *L. pennellii* introgression lines (ILS). These introgression lines are a set of purebred lines each containing a small chromosome segment of the wild green-fruited *Lycopersicon pennellii* in the background -

of the cultivated *L. esculentum* cv M-82 (Eshed et al., 1992, Theor Appl. Genet., 83:1027-1034). These lines were developed from an initial interspecific cross between *L. pennellii* and *L. esculentum* cv M-82. The resulting F1 individuals were backcrossed to *L. esculentum* cv M-82 and selfed for several generations. During the process, chromosome segments of *L. pennellii* were selected for using restriction fragment length polymorphism probes covering the entire tomato genome. The introgression lines therefore provide a set of nearly-isogenic lines for segments of the wild-species genome and enable the association of yield traits with specific wild-species chromosome segments (Eshed Y. and Zamir D. 1994. Theor Appl. Genet., 88:891-897). Eleven such introgression lines were used for this study. The assumption was that crossing the 904 high starch line with this broad spectrum of genotypes, and crossing in parallel M-82 with the same identical genotypes would supply us with a broad spectrum of genetic background in which the genetic effect of 904 could be discerned.

Starch levels of the immature fruit, as well as soluble solids levels of the mature fruit, from the average of the eleven hybrids with line 904 were significantly higher than starch levels of immature fruit and soluble solids levels from mature fruit from the parallel hybrids with M-82 (Table 2). A number of these immature fruit, representing the high starch hybrids with 904 and the low starch hybrids with M-82 were subjected to a detailed enzymatic analysis of the enzymes involved in sucrose to starch metabolism in the immature tomato fruit (as described above). Table 3 shows that of the ten enzymes assayed, only ADPGPPase activity was significantly higher in the hybrids with the high starch line (904), compared to the hybrids with the M-82 line.

Table 1: Starch levels and enzyme activities of immature tomato fruit (approximately 15 DAA) for CV M-82 and three high starch breeding lines 900, 901 and 904.

	M-82	900	901	904
Starch (mg/gfw)	13.1	23.3 *	23.2 *	34.9 *
Enzymes (nmol/gfw/min)				
Invertase	15480	14690	18980	17870
Sucrose synthase	29570	31970	33260	27570
fructokinase	91	150 *	92	137
phosphoglucomutase	5760	6650	7830	7490
phosphoglucosisomerase	1950	2000	2870	2060
UDPglu PPase	15080	16760	17250	14760
ADPglu PPase	40	142 *	84 *	268 *

*Indicates statistical significance ($P < 0.05$) of each individual high starch line as compared to M-82.

Table 2: Starch content of immature fruit (approx. 15 days after anthesis) and °Brix (TSS) values of mature fruit of line 904, M-82, the hybrid between them, the mix of 11 hybrids between M-82 and 11 introgression lines (ILS) and the mix of 11 hybrids between 904 and the same 11 ILS.

Genotype	Starch mg/gfw	°Brix
M-82	23 b	4.1 b
904	58 a	8.1 a
M-82 x 904	46 a	7.1 a
M-82 x ILS	25 b	5.3 b
904 x ILS	44 a	7.5 a

Letters signify statistical significance at $P < 0.05$

Table 3: Activities of enzymes in the sucrose to starch metabolic pathway in immature tomato fruit.

	Activity (nmol/gfw/min)		
Enzyme	904 x ILS	M-82 x ILS	Ratio
Invertase	520	620	0.83
Sucrose synthase	710	560	1.27
fructokinase	225	219	1.03
glucokinase	23	25	0.94
phosphoglucomutase	6900	5340	1.31
phosphoglucoisomerase	3160	2630	1.21
UDPglu PPase	8490	7130	1.19
ADPglu PPase	190	56	3.67*
starch synthase, sol.	48	38	1.26
starch synthase, insol.	5	5	0.93

* statistical significance at $P < 0.05$

To further study the genetic trait for high ADPGPPase activity in immature fruit, specific DNA primers for the genes for the four ADPGPPase subunits (Chen and Janes, 1997 and Park and Cheung, 1998) were devised which could distinguish between the *L. hirsutum* derived gene and the *L. esculentum* derived gene, as described in the following paragraph.

PCR analysis of ADPGPPase subunits

Amplification reactions of the ADPGPPase subunits (25 µl final volume) contained 10 ng template DNA, 25 mM TAPS (pH=9.3 at 25°C), 50 mM KCl, 2.5 mM $MgCl_2$, 1 mM (mercaptoethanol, 0.2 mM of each of the four deoxyribonucleotide triphosphates (dATP, dCTP, dGTP and dTTP), 10 ng of each of the 2 primers (forward and reverse primers, see Table 4), and 1 unit of thermostable Taq DNA polymerase (SuperNova Taq polymerase, Madi Ltd., Rishon Le Zion, Israel). Reactions were carried out in an automated thermocycler (MJ Research Inc., Watertown, Massachusetts, USA). Initial incubation was at 94°C for 1 min, followed by 34 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and polymerization at 72°C for 1 min and 45 sec. Final polymerization at 72°C was carried out for

7 min after cycles were completed. 10 µl of the amplification products were digested with 15 units of the restriction endonuclease found to generate the *L. hirsutum* specific alleles (Table 4). Digestions were carried out according to the manufacturer recommendations (New England Biolabs Inc., Beverly, MA, USA). The digestion products were visualized by electrophoresis in 1.2% agarose gel and detected by staining with ethidum bromide.

Line 904 was shown to carry the *L. hirsutum* gene for large subunit 1 (LS1) while the other subunits of ADPGPPase in line 904 were shown to be derived from the *L. esculentum.*

In order to show that the *L. hirsutum* derived LS1 was correlated with increased ADPGPPase activity and increased starch level in the immature fruit, an F2 population of 64 plants of the cross between the high starch line 904 and the standard line M-82 was grown. The plants were genotypically typed at the first true leaf stage to determine whether they were homozygous for the *L. hirsutum* ADPGPPase LS1 allele (HH), homozygous for the *L. esculentum* allele (EE) or heterozygous (HE) containing both alleles. The 64 F2 plants segregated for the LS1 in a ratio of 16:31:17, as expected for a single locus. Immature fruit from a minimum of 4 of each of the determined F2 genotypes were assayed for starch levels and for ADPGPPase activity. Results are presented in Table 5 and clearly show that the *L. hirsutum* allele for ADPGPPase LS1, as characterized by the specific PCR primers described, is associated with increased ADPGPPase activity in the immature fruit. Furthermore, the TSS values of the mature fruit was similarly influenced by the genotype of the LS1 gene.

Table 4. Forward and reverse primers used in the PCR analysis of the 3 large and 1 small subunits of ADPGPPase and the restriction endonuclease used to digest the PCR product in order to obtain the *L. Hirsutum* specific allele.

ADPGPPase Subunit	Forward primer	Reverse primer	Restriction endonuclease
Large (LS1)	GTTCATTTGGGGA GAGTGAGCAC	GGGCAGCAGAAT TGTACTGTGTC	Hinf I
Large (LS2)	CTATTGGTGGTTG TTACCGGGT	CACTGTTCCAATA TCCTCCCAG	Hinf I
Large (LS3)	GCATATTGCTCGT GCGTACAAC	CTTTTCGCTGAAG GACATGACC	-
Small	TTTCGTCTTCTCA TCTCGCCGGA	GGCGATTTAGAG AGGCAGAGTTG	RsaI

Table 5: Effect of genotype of LS1 on ADPGPPase activity and starch levels in immature fruit and TSS in mature fruit. ADPGPPase activity and starch levels are the averages from 4 fruit (8-13 gr.) from individual F2 plants. TSS values are the average of a minimum of 5 fruit of each genotype.

Genotype	ADPGPPase	Starch	TSS
EE	104 c	16.4 b	5.3 b
EH	306 b	25.2 ab	5.9 ab
HH	450 a	37.3 a	6.3 a

Letters signify statistical difference at $P < 0.05$

Sequencing of the gene encoding ADPGPPase large subunit (LS1) from *L. hirsutum*.

Total RNA was extracted from young fruits (3 grams in weight) of an individual plant homozygous for the ADPGPPase large subunit (LS1). The RNA extraction was carried out using the TRIzol reagent system (GibcoBRL life technologies, Gaithersburg, MD, USA). The total RNA was used as template for first strand cDNA synthesis using the Superscript preamplification system (GibcoBRL life technologies, Gaithersburg, MD. USA). The cDNA prepared was used as template in a PCR reaction to amplify the gene encoding ADPGPPase large subunit (LS). The DNA fragments containing the ADPGPPase large subunit (LS) were excised from an agarose gel and purified using the GENECLEAN II kit (BIO 101 inc., La Jolla CA, USA). The PCR bands were then cloned into an pGEM-T Easy vector using the pGEM-T and pGEM-T Easy Vector Systems according to the manufacturer recommendations (Promega corporation, Madison, WI, USA). The DNA clones were sequenced using an automated sequencer (Applied Biosystems, Foster City, CA, USA).

Table 6 is the nucleotide sequence of ADPGPPase LS1 (ADPGlucose pyrophosphorylase, large subunit 1) from *L.hirsutum*. Table 7 is the derived amino acid sequence for ADPGPPase LS1 (ADPGlucose pyrophosphorylase, large subunit 1) from *L. hirsutum*.

Table 6: Nucleotide sequence of ADPGPPase LS1 (ADPGlucose pyrophosphorylase, large subunit 1) from *L. hirsutum*

```
   1 ATGAAATCGA CGGTTCATTT GGGGAGAGTG AGCACTGGTG CTTTAACAA
  51 TGGAGAGAAG GAGATTTTTG GGGAGAAGAT GAGAGGGAGT TTGAACAACA
 101 ATCTCAGGAT TAATCAGTTG TCGAAAAGTT TGAAACTTGA GAAGAAGGAG
 151 AAGAAGATTA AACCTGGGGT TGCTTACTCT GTGATCACTA CTGAAAATGA
 201 CACAGAGACT GTGTTCGTAG ATATGCCACG TCTTGAGAGA CGCCGGGCAA
 251 ATCCCAAGGA TGTGGCTGCA GTCATATTAG GAGGAGGCGA AGGGACCAAG
 301 TTATTCCCAC TTACAAGTAG AACTGCAACC CCTGCTGTTC CGGTTGGAGG
 351 ATGCTACAGG CTCATAGACA TCCCGATGAG CAACTGTATC AACAGTGCTA
 401 TTAACAAGAT TTTTGTGCTG ACACAGTACA ATTCTGCTGC CCTGAATCGT
 451 CACATTGCTC GAACGTATTT TGGCAATGGT GTGAGCTTTG GAGATGGATT
 501 TGTCGAGGTA CTAGCTGCAA CTCAGACACC TGGGGAAGCA GGAAAAAAAT
 551 GGTTTCAAGG AACAGCAGAT GCTGTCAGAA AATTTATATG GGTTTTTGAG
 601 GACGCTAAGA ACAAGAATAT TGAAAATATC CTTGTATTAT CTGGGGATCA
 651 TCTTTATAGG ATGGATTATA TGGAGTTGGT GCAGAACCAT ATTGACAGAA
 701 ATGCTGATAT TACTCTTTCA TGTGCACCAG CTGAGGACAG CCGAGCATCA
 751 GATTTTGGGC TGGTCAAGAT TGACAGCAGA GGCAGAGTTG TCCAGTTTGC
 801 TGAAAAACCA AAAGGTTTTG AGCTTAAAGC AATGCAAGTA GATACTACTC
 851 TTGTTGGATT ATCTCCACAA GATGCGAAGA AATCCCCTTA TATTGCTTCA
 901 ATGGGAGTTT ATGTTTTCAA GACAGATGTA TTGCTGAAGC TCTTGAAATG
 951 GAGCTACCCC ACTTCTAATG ATTTTGGCTC TGAAATTATA CCAGCAGCTA
1001 TTGATGATTA CAATGTCCAA GCATACATTT TCAAAGACTA TTGGGAGGAC
1051 ATTGGAACAA TTAAATCTTT CTATAATGCT AGCTTGGCGC TCACACAAGA
1101 GTTTCCAGAG TTCCAATTTT ATGATCCAAA AACACCTTTT TACACATCTC
1151 CTAGGTTCCT TCCACCAACC AAGATAGACA ATTGCAAGAT TAAGGATGCC
1201 ATAATTTCTC ATGGATGTTT CTTGCGAGAT TGCTCTGTGG AACACTCCAT
1251 AGTGGGTGAA AGATCACGCT TAGACTGTGG TGTTGAACTG AAGGATACTT
1301 TCATGATGGG AGCAGACTAC TACCAAACAG AATCTGAGAT TGCCTCCCTG
1351 TTAGCAGAGG GGAAAGTACC GATTGGGATT GGGGAAAATA CAAAAATAAG
1401 GAAATGTATC ATTGACAAGA ACGCAAAGAT AGGAAAAAAT GTTTCAATCA
1451 TTAATAAAGA TGGTGTTCAA GAGGCAGACC GACCAGAGGA AGGATTCTAC
1501 ATACGATCAG GGATAACCAT TATATCAGAG AAAGCCACAA TTAGAGATGG
1551 AACAGTTATA TGA
```

Table 7: Derived amino acid sequence for ADPGPPase LS1 from *L. hirsutum*

```
MKSTVHLGRVSTGGFNNGEKEIFGEKMRGSLNNNLRINQL
SKSLKLEKKEKKIKPGVAYSVITTENDTETVFVDMPRLERRRAN
PKDVAAVILGGGEGTKLFPLTSRTATPAVPVGGCYRLIDIPMSNC
INSAINKIFVLTQYNSAALNRHIARTYFGNGVSFGDGFVEVLAAT
QTPGEAGKKWFQGTADAVRKFIWVFEDAKNKNIENILVLSGDHL
YRMDYMELVQNHIDRNADITLSCAPAEDSRASDFGLVKIDSRGR
VVQFAEKPKGFELKAMQVDTTLVGLSPQDAKKSPYIASMGVYV
FKTDVLLKLLKWSYPTSNDFGSEIIPAAIDDYNVQAYIFKDYWED
IGTIKSFYNASLALTQEFPEFQFYDPKTPFYTSPRFLPPTKIDNCKI
KDAIISHGCFLRDCSVEHSIVGERSRLDCGVELKDTFMMGADYY
QTESEIASLLAEGKVPIGIGENTKIRKCIIDKNAKIGKNVSIINKDG
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VQEADRPEEGFYIRSGITIISEKATIRDGTVI

In the foregoing example, the large subunit 1 of ADPGPPase was shown to increase starch level. Although not specifically tested, it is reasonable to assume that the present invention can also be carried out by transferring the *L. hirsutum* genes for any of the other 3 subunits of the enzyme, using the specific PCR markers developed for each of these genes, as they may also increase starch. In addition, transfer of ADPGPPase genes from other wild tomato species, other than *L. hirsutum*, may also increase starch in crosses with *L. esculentum*. Additionally, transfer of genes for other enzymes of starch synthesis from wild species, such as fructokinase and sucrose synthase for which the gene sequences from *L. esculentum* are known, may also increase starch levels.

Those skilled in the art will recognize that the described gene can be used to genetically transform plants to increase starch content. Plants that can genetically be transformed to have increased starch content include a large range of agriculturally important crops, such as but not limited to, potato, tomato, corn, wheat, cotton, banana, soybean, pea and rice. The plant transformation technology, including methods of transformation, such as the use of *Agrobacterium tumefaciens*, and methods of developing constructs, including the use of tissue specific promoters is well established and has recently been reviewed by Christou, P. ("Transformation technology", Trends in Plant Science, 1:423-431). There are presently available numerous promoters, including the constitutive promoters (CaMV) 35S and the maize ubiquitin promoter. In addition, there are, for example, organ/tissue specific promoters, for expression in seeds, tubers, immature fruit, mature fruit, pollen, roots and other organs.

The above examples are provided to better elucidate the practice of the present invention and should not be interpreted in any way to limit the scope of the present invention. Those skilled in the art will recognize that various modifications can be made to the methods described herein while not departing from the spirit and scope of the present invention.

CLAIMS

What is claimed is:

1. A method for controlling starch synthesis in tomatoes comprising:

providing a population of plants derived from interspecific crosses of *Lycopersicon* spp. with *Lycopersicon esculentum* genotypes; and

selecting individuals of said population that each contain an allele of a gene that increases starch synthesis, said gene originating from said *Lycopersicon* spp.

2. The method according to claim 1 wherein said step of selecting comprises selecting individuals that each contain the allele of the gene that encodes for an enzyme that catalyzes a metabolic step in starch synthesis.

3. The method according to claim 1 wherein said step of selecting comprises selecting individuals that each contain the allele of the gene that encodes for a subunit of ADPGPPase.

4. The method according to claim 1 wherein said step of selecting comprises selecting individuals that each contain the allele of the gene that encodes for a *Lycopersicon hirsutum*-derived subunit of ADPGPPase.

5. The method according to claim 1 wherein said step of selecting comprises selecting by using a molecular marker for said gene.

6. The method according to claim 5 wherein said molecular marker comprises step of selecting comprises a *Lycopersicon hirsutum*-derived large subunit (LS1) of ADPGPPase.

7. The method according to claim 2 wherein said step of selecting comprises selecting by measuring activity of said enzyme in young fruit and selecting those young fruit with high activity of said enzyme.

8. The method according to claim 2 wherein said step of selecting comprises selecting by measuring ADPGPPase activity of said young fruit, and selecting those young fruit with high ADPGPPase activity.

9. The method according to claim 1 wherein said *Lycopersicon* spp. comprises a *Lycopersicon* spp. of green-fruited *Eriopersicon* subgenus.

10. The method according to claim 1 wherein said *Lycopersicon* spp. comprises *Lycopersicon hirsutum*.

11. A method of producing genetically transformed plants which have elevated starch content, comprising the steps of:

a) inserting into the genome of a plant cell a recombinant double stranded DNA

molecule comprising

(i) a selected promoter

(ii) a structural DNA sequence that causes the production of an RNA sequence which encodes the above described ADPGPPase LS1 protein

b) obtaining transformed plant cells

c) regenerating from the transformed plant cells genetically transformed plants with elevated starch content.

12. The method according to claim 11 wherein said plant cell is selected from the group consisting of a tomato cell, a potato cell, a cell from a solanaceous plant, a legume cell, and a grain crop cell.

13. The method according to claim 11 wherein said promoter is selected from the group consisting of an immature fruit promoter, a tuber promoter, and a seed promoter.

14. The method according to claim 11 wherein said step of regenerating comprises regenerating genetically transformed plants with elevated starch content in an immature fruit.

15. The method according to claim 11 wherein said step of regenerating comprises regenerating genetically transformed plants with elevated starch content in a tuber.

16. The method according to claim 11 wherein said step of regenerating comprises regenerating genetically transformed plants with elevated starch content in a seed.

17. A method according to claim 1 and additionally comprising the step of propagating said individuals of said population.

18. A method according to claim 17 wherein the step of propagating includes the step of vegetative propagation.

19. A method according to claim 17 wherein the step of propagating includes the step of propagation by seed.

20. A method according to claim 11 and additionally comprising the step of propagating said genetically transformed plants.

21. A method according to claim 20 wherein the step of propagating includes the step of vegetative propagation.

22. A method according to claim 20 wherein the step of propagating includes the step of propagation by seed.

23. A plant produced according to the method of claim 1.

24. A plant produced according to the method of claim 11.

25. A fruit produced by a plant in accordance with claim 23.

26. A fruit produced by a plant in accordance with claim 24.

27. A seed which when grown yields a plant in accordance with claim 23.

28. A seed which when grown yields a plant in accordance with claim 24.

29. A gene that controls sucrose-starch metabolism comprising a nucleotide sequence as follows:

```
1    ATGAAATCGA CGGTTCATTT GGGGAGAGTG AGCACTGGTG CTTTAACAA
51   TGGAGAGAAG GAGATTTTTG GGGAGAAGAT GAGAGGGAGT TTGAACAACA
101  ATCTCAGGAT TAATCAGTTG TCGAAAAGTT TGAAACTTGA GAAGAAGGAG
151  AAGAAGATTA AACCTGGGGT TGCTTACTCT GTGATCACTA CTGAAAATGA
201  CACAGAGACT GTGTTCGTAG ATATGCCACG TCTTGAGAGA CGCCGGGCAA
251  ATCCCAAGGA TGTGGCTGCA GTCATATTAG GAGGAGGCGA AGGGACCAAG
301  TTATTCCCAC TTACAAGTAG AACTGCAACC CCTGCTGTTC CGGTTGGAGG
351  ATGCTACAGG CTCATAGACA TCCCGATGAG CAACTGTATC AACAGTGCTA
401  TTAACAAGAT TTTTGTGCTG ACACAGTACA ATTCTGCTGC CCTGAATCGT
451  CACATTGCTC GAACGTATTT TGGCAATGGT GTGAGCTTTG GAGATGGATT
501  TGTCGAGGTA CTAGCTGCAA CTCAGACACC TGGGGAAGCA GGAAAAAAAT
551  GGTTTCAAGG AACAGCAGAT GCTGTCAGAA AATTTATATG GGTTTTTGAG
601  GACGCTAAGA ACAAGAATAT TGAAAATATC CTTGTATTAT CTGGGGATCA
651  TCTTTATAGG ATGGATTATA TGGAGTTGGT GCAGAACCAT ATTGACAGAA
701  ATGCTGATAT TACTCTTTCA TGTGCACCAG CTGAGGACAG CCGAGCATCA
751  GATTTTGGGC TGGTCAAGAT TGACAGCAGA GGCAGAGTTG TCCAGTTTGC
801  TGAAAAACCA AAAGGTTTTG AGCTTAAAGC AATGCAAGTA GATACTACTC
851  TTGTTGGATT ATCTCCACAA GATGCGAAGA AATCCCCTTA TATTGCTTCA
901  ATGGGAGTTT ATGTTTTCAA GACAGATGTA TTGCTGAAGC TCTTGAAATG
951  GAGCTACCCC ACTTCTAATG ATTTTGGCTC TGAAATTATA CCAGCAGCTA
1001 TTGATGATTA CAATGTCCAA GCATACATTT TCAAAGACTA TTGGGAGGAC
1051 ATTGGAACAA TTAAATCTTT CTATAATGCT AGCTTGGCGC TCACACAAGA
1101 GTTTCCAGAG TTCCAATTTT ATGATCCAAA AACACCTTTT TACACATCTC
1151 CTAGGTTCCT TCCACCAACC AAGATAGACA ATTGCAAGAT TAAGGATGCC
1201 ATAATTTCTC ATGGATGTTT CTTGCGAGAT TGCTCTGTGG AACACTCCAT
1251 AGTGGGTGAA AGATCACGCT TAGACTGTGG TGTTGAACTG AAGGATACTT
1301 TCATGATGGG AGCAGACTAC TACCAAACAG AATCTGAGAT TGCCTCCCTG
1351 TTAGCAGAGG GGAAAGTACC GATTGGGATT GGGGAAAATA CAAAAATAAG
1401 GAAATGTATC ATTGACAAGA ACGCAAAGAT AGGAAAAAAT GTTTCAATCA
1451 TTAATAAAGA TGGTGTTCAA GAGGCAGACC GACCAGAGGA AGGATTCTAC
1501 ATACGATCAG GGATAACCAT TATATCAGAG AAAGCCACAA TTAGAGATGG
1551 AACAGTTATA TGA
```

30. A protein that controls sucrose-starch metabolism comprising a derived amino acid sequence as follows:

```
MKSTVHLGRVSTGGFNNGEKEIFGEKMRGSLNNNLRINQL
SKSLKLEKKEKKIKPGVAYSVITTENDTETVFVDMPRLERRRAN
PKDVAAVILGGGEGTKLFPLTSRTATPAVPVGGCYRLIDIPMSNC
INSAINKIFVLTQYNSAALNRHIARTYFGNGVSFGDGFVEVLAAT
QTPGEAGKKWFQGTADAVRKFIWVFEDAKNKNIENILVLSGDHL
YRMDYMELVQNHIDRNADITLSCAPAEDSRASDFGLVKIDSRGR
```

VVQFAEKPKGFELKAMQVDTTLVGLSPQDAKKSPYIASMGVYV
FKTDVLLKLLKWSYPTSNDFGSEIIPAAIDDYNVQAYIFKDYWED
IGTIKSFYNASLALTQEFPEFQFYDPKTPFYTSPRFLPPTKIDNCKI
KDAIISHGCFLRDCSVEHSIVGERSRLDCGVELKDTFMMGADYY
QTESEIASLLAEGKVPIGIGENTKIRKCIIDKNAKIGKNVSIINKDG
VQEADRPEEGFYIRSGITIISEKATIRDGTVI

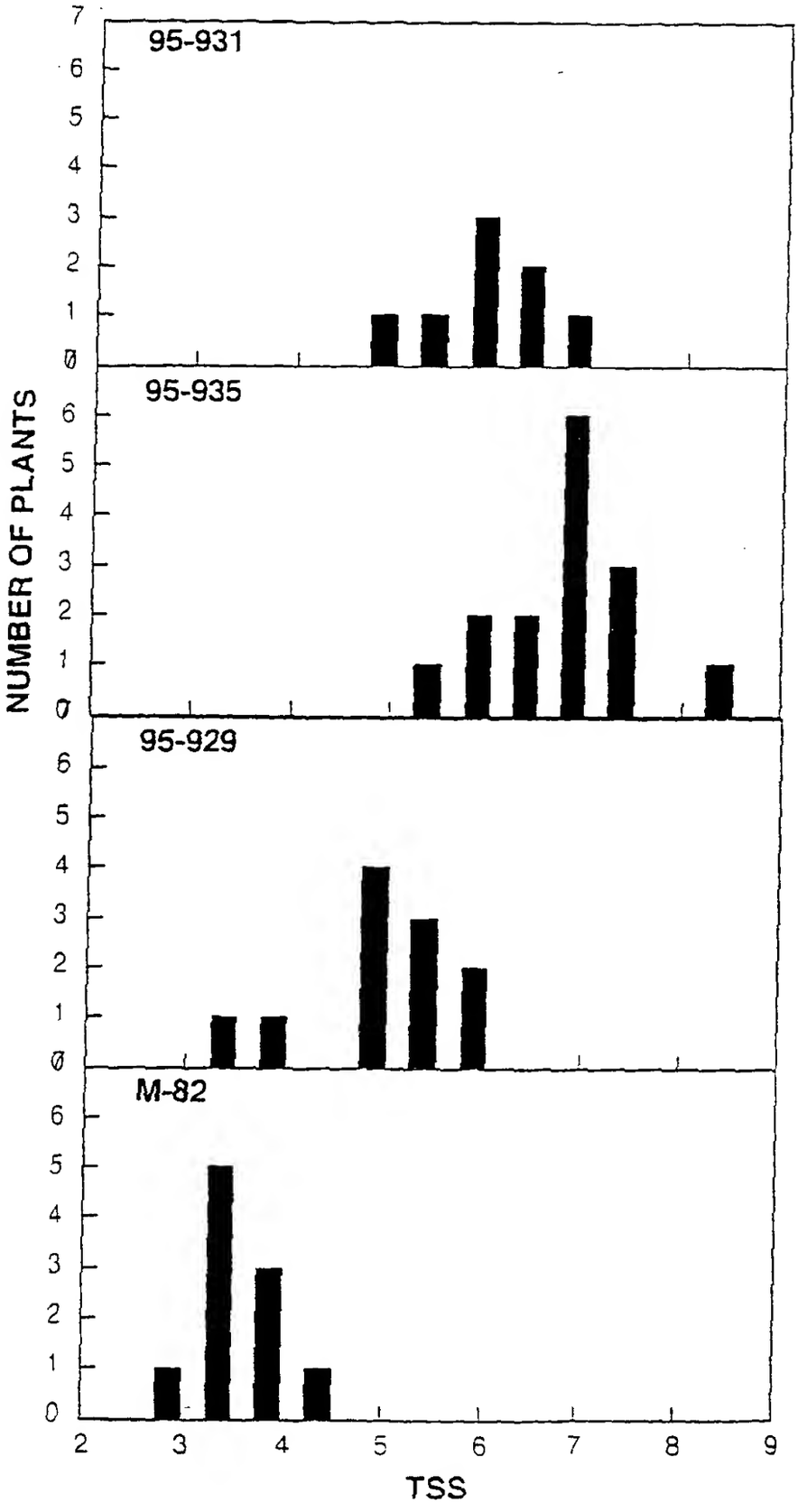

FIG. I

INTERNATIONAL SEARCH REPORT

[redacted] ional Application No PCT/IL 99/00396 U 013220-5

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/82 C12N15/54 C12N9/12 C12N5/10 C12Q1/68
A01H1/04 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Date of the actual completion of the international search	Date of mailing of the international search report
25 May 2000	14/06/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Holtorf, S

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Information on patent family members

International Application No
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(30) Priority Data:
125425 20 July 1998 (20.07.98) IL

(71) Applicant *(for all designated States except US)*: STATE OF ISRAEL–MINISTRY OF AGRICULTURE [IL/IL]; Volcani Research Center, P.O. Box 6, 50250 Beit Dagan (IL).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: SCHAFFER, Arthur, A. [IL/IL]; Hazayit Street 16, 73127 Hashmonaim (IL). LEVIN, Ilan [IL/IL]; Eshel Street 8, 76804 Mazkeret Batya (IL). PETREIKOV, Marina [IL/IL]; Bernstein Street 55/22, 75000 Rishon le Zion (IL). BAR, Moshe [IL/IL]; Nahal Soreq Street 10, 75246 Rishon le Zion (IL).

(74) Agents: COLB, Sanford, T. et al.; Sanford T. Colb & Co., P.O. Box 2273, 76122 Rehovot (IL).

(81) Designated States: AE, AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, EE (Utility model), ES, FI, FI (Utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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(54) Title: CONTROLLING STARCH SYNTHESIS

(57) Abstract

A method for controlling starch synthesis in tomatoes including providing a population of plants derived from interspecific crosses of *Lycopersicon* spp. with *Lycopersicon esculentum* genotypes, and selecting individuals of the population that each contain an allele of a gene that increases starch synthesis, the gene originating from the *Lycopersicon* spp.

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INTERNATIONAL SEARCH REPORT

Inte /onal Application No
PCT/IL 99/00396

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/82 C12N15/54 C12N9/12 C12N5/10 C12Q1/68
A01H1/04 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

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